SOME ASPECTS OF CITRIC ACID METABOLISM*

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(Received for publication, July 12, 1933)

In addition to its presence in milk (9) and urine (1) citric acid has, within the past few years, been found in many other body fluids including sweat (12), cerebrospinal fluid (2), aqueous humor (7), follicular fluid (14), and blood (3). Apparently, saliva is the only body fluid tested in which citrate has not been detected.[1]

Although citric acid appears to be so generally distributed and is excreted by the normal individual in amounts from 0.2 to 1.0 gm. daily (15), its rôle in metabolism is obscure. Tolerance studies have led to confusing results because of the interfering action of the large amounts of alkali present in the citrates used (sodium citrate contains practically as much alkali per gm. as sodium bicarbonate). The fact that citric acid forms a soluble, slightly ionized salt with calcium has suggested that it may be related to calcium metabolism (5), but Greenberg and Greenberg (6) find little good evidence that such a slightly ionized combination is present in the blood. In 1931, Östberg (15), on the basis of evidence which showed that the amount of citric acid excreted varies directly with the pH of the urine, suggested that citric acid is primarily a part of the acid-base regulatory mechanism and that it is synthesized to neutralize excess alkali just as ammonia is synthesized to neutralize excess acid. On the other hand, the large amounts of citrate excreted in fasting and on ketogenic diets (4) indicate that citrate metabolism must be controlled by other factors in addition to acid-base relationships.

In the present work, the influence of acid-base relationships and

* A report of this work was presented before the American Society of Biological Chemists at Cincinnati, April 10, 1933 (*J. Biol. Chem.*, 100, lxi (1933)).

[1] The methylene blue-enzyme method failed to show even a trace.

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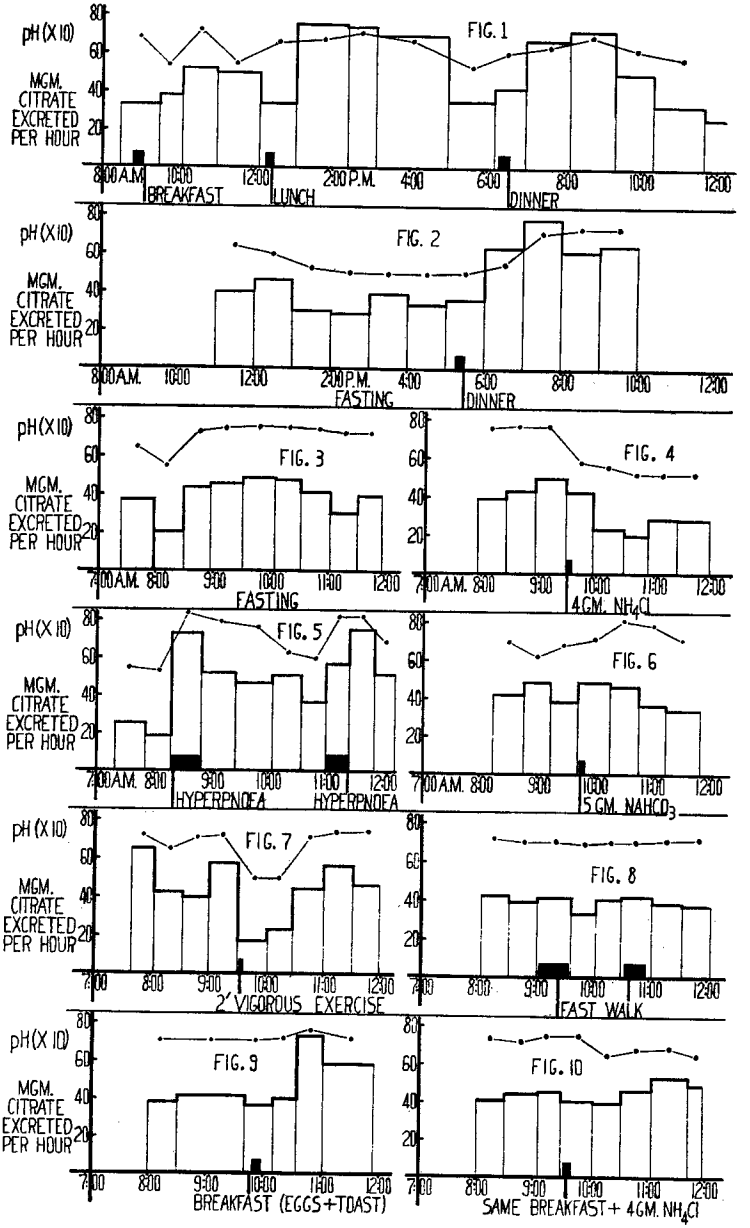
of dietary factors was investigated by following blood citrate concentration and urinary excretion of citric acid after exercise and after the administration of food, sodium bicarbonate, ammonium chloride, and citric acid itself. The pentabromoacetone method[2] was used for the determination of citrate in urine, the Thunberg methylene blue method[3] for the analysis of blood.

Analysis of urines collected in half hour periods throughout the day from a human subject on a mixed diet revealed that the rate of citrate excretion is directly related to the ingestion of food and

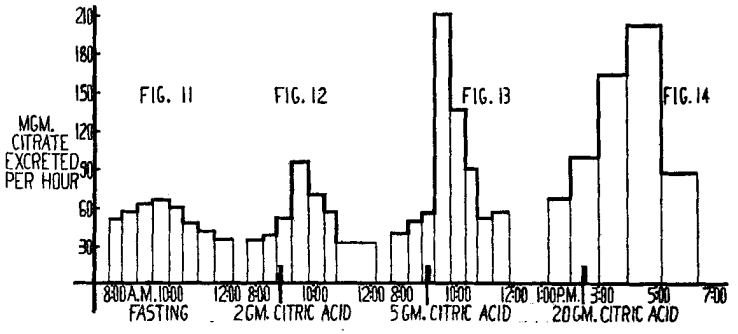
[2] Numerous modifications were introduced into the original Amberg-McClure method (1). The urine sample (50 cc., or smaller amounts diluted to that volume) to which 10 cc. of sulfuric acid (1:1) and 3 cc. of 37.5 per cent potassium bromide solution are added, is heated on a water bath to 52–54° (the limit of 50–55° is rather too wide (10)); sufficient 5 per cent potassium permanganate is added immediately to give a precipitate of manganese dioxide; this is usually 15 cc., but more must be used when lactic acid or other easily oxidized substances are present. An excess of acidified concentrated ferrous sulfate solution is added to reduce the precipitated manganese dioxide and the flask is allowed to cool overnight in the ice box (8). The solution is filtered cold through a Gooch crucible and washed with several small portions of water. The crucible is dried in a sulfuric acid desiccator for 24 hours, weighed, heated at 105° for 10 hours, weighed again, and the difference in weight taken as the weight of pentabromoacetone (13). The amount of citric acid (plus 1 molecule of water) present in the original sample is calculated from the formula, $1.05\ (0.464\ P + 0.019\ S)$, where P is the weight of pentabromoacetone in mg., 0.019 is a solubility factor, and S is the volume of the discarded filtrate (8). Control experiments showed that preliminary treatment with bromine and charcoal (13) did not change the citrate values obtained on normal human urine. Duplicate analyses on standard solutions and on urine samples checked consistently within a few per cent.

[3] Certain modifications of Thunberg's method (17) have already been described (11). Seeds (usually 1 gm. of peeled seeds to 9 cc. of 0.87 per cent K_2HPO_4) were mechanically ground, vigorously, for a period of ½ hour, then centrifuged at high speed for an hour, and allowed to stand at room temperature for at least another hour before use. This procedure produced an extract which maintained a fairly constant activity for 3 hours at room temperature, or longer if kept cold, with very little change in the "critical value." The interfering action of inorganic salts, especially calcium, in the estimation of very small amounts of citrate as found in blood, was minimized by the use of the extract thus prepared; furthermore the use of 1:60,000 methylene blue instead of 1:30,000, as suggested by Thunberg, permitted the precise determination of as little as 2 mg. of citrate per 100 cc. of blood with no interference on the part of the serum salts.



FIGS. 1 TO 10. The relation between urinary pH and citrate excretion

that it appears to parallel the pH of the urine during these short periods as well as in the 24 hour periods reported by Östberg. Following each meal, notwithstanding the absence of food known to contain citrate, such as milk and citrus-fruits, there was a marked increase in the rate of citric acid excretion along with an increase in the pH of the urine as shown in Fig. 1. In Fig. 2 when no breakfast or lunch was eaten both the pH and rate of excretion of citrate remained low until dinner; then they both increased. The experiments recorded in Figs. 3 to 6 were designed to elucidate the relation between urinary pH and citrate excretion. Fig. 3 shows the rate of citrate excretion during a morning's fast. The ingestion of 4 gm. of ammonium chloride (Fig. 4)



FIGS. 11 TO 14. Influence of ingestion of citric acid on citrate excretion

during a similar fasting period caused a decrease in both the pH of the urine and the rate of citrate excretion. Two periods of voluntary hyperpnea and the accompanying alkalosis (Fig. 5) caused marked increases in the rate of citrate excretion. Administration of 5 gm. of sodium bicarbonate (Fig. 6) proved insufficient to cause a significant change in citrate excretion although it increased the pH of the urine. The depressing action of ammonium chloride and the accelerating action of voluntary hyperpnea indicate that the mechanism which controls the output of citrate is flexible like that for ammonia production and that citrate may take an active part in maintaining the proper acid-base equilibria.

Strenuous exercise caused a marked decrease in the rate of citrate excretion (Fig. 7) but two periods of mild exercise (walking, Fig. 8) apparently had no effect. The marked decrease following

strenuous exercise may be related to the accumulation of large amounts of lactic acid and the consequent greater acidity of the urine.

These almost parallel changes in citrate excretion and pH of urine produced by alkalinizing or acidifying measures suggest that the increased citrate output following meals might be simply a reflection of the alkaline tide. However, ingestion of food must act also in another way, inasmuch as sodium bicarbonate produced a more alkaline urine than eggs and toast (Fig. 9) but caused little change in citrate output. Furthermore, when ammonium chloride was added to a breakfast of eggs and toast (Fig. 10) there

TABLE I

Influence of Fasting and Ingestion of $NaHCO_3$ on Citrate Content of Rabbit Blood

Day	Time	Procedure	Alkali reserve	Blood citrate
			vol. per cent	*mg. per cent*
1	2.30 p.m.	Food removed		
	3.30 "	Heart puncture	44.9	13.8
2	10.30 a.m.	" "	51.3	4.7
	2.30 p.m.	0.75 gm. $NaHCO_3$ per kilo		
	9.30 "	Heart puncture	61.7	5.0
3	10.30 a.m.	" "	62.0	3.3
4	10.40 "	" "	57.9	3.1
	10.50 "	1.5 gm. $NaHCO_3$ per kilo		
	4.10 p.m.	Heart puncture	82.0	4.7

was still an increase in the rate of citrate excretion in spite of a lowered pH of the urine. Since 50 gm. of glucose caused little change in citrate output, the increase following eggs and toast which is not referable to alkaline tide may be associated with the metabolism of fat or protein. Boothby and Adams (4) suggested that citrate excretion is particularly related to fat and protein metabolism when they found that large amounts of citric acid were excreted at times when the R.Q. indicated that only fat and protein were being oxidized.

Citrate excretion could scarcely be influenced by unsuspected traces of citrate in food. In Figs. 11 to 14, 2 gm. of citric acid taken during a fasting period increased the urinary citrate by less

than 30 mg., 5 gm. by less than 125 mg., and 20 gm. by less than 350 mg. In each case, from 1.5 to 2.5 per cent of the ingested acid escaped oxidation. In a 40 day experiment in which the subjects took a standardized weighed diet and in which 24 hour urine samples were analyzed, similar increases were noted after the ingestion of citric acid; in some cases these were followed by a compensatory decrease in excretion on the following day. Such a compensatory decrease occurring within the same 24 hour period may explain why Östberg in numerous instances missed the increased citrate excretion after the ingestion of citric acid.

TABLE II

Influence of Ingestion of $NaHCO_3$ and NH_4Cl on Blood Citrate Concentration

Subject	Time	Procedure	Alkali reserve	Blood citrate
			vol. per cent	*mg. per cent*
Rabbit B	10.00 a.m.	Food removed		
	11.15 "	Heart puncture	48.8	7.6
	11.40 "	1.25 gm. $NaHCO_3$ per kilo		
	2.25 p.m.	Heart puncture	75.5	7.6
	5.30 "	" "	88.3	7.1
Rabbit C	10.00 a.m.	Food removed		
	11.35 "	Heart puncture	64.0	7.8
	11.50 "	0.75 gm. NH_4Cl per kilo		
	2.40 p.m.	Heart puncture	39.6	5.8
	5.45 "	" "	39.2	3.9

In order to visualize more clearly the events in citric acid metabolism, an attempt was made to correlate the changes in urinary excretion of citric acid with the citric acid content of human and rabbit blood, under the action of the same variables. In a typical experiment on a well fed rabbit (Table I) the blood citrate was 13.8 mg. per 100 cc. A 20 hour fast reduced this value to 4.7 mg. Administration of 0.75 gm. of sodium bicarbonate by stomach tube resulted after 7 hours in a very slight increase of blood citrate, to 5 mg. Although the total carbonic acid remained at about 60 volumes per cent, further fasting caused the blood citrate to decline to 3.1 mg. A second administration of bicarbonate raised the total carbonic acid to 82 volumes per cent, the blood citrate to 4.7 mg. per cent. In another experiment (Table II) sodium bi-

carbonate was administered to a well fed rabbit immediately after food had been removed; within 7 hours, in spite of a high carbon dioxide-combining power, the blood citrate decreased rather than increased. Another fasting rabbit was given ammonium chloride. The combined action of fasting and acid served to reduce the blood citrate to 3.9 mg. within 7 hours. Fasting thus decreased the blood citrate of rabbits regardless of acid or alkaline conditions and the changes produced by administration of acid or base were

TABLE III

Citrate Content of Blood Taken from Various Blood Vessels of the Rabbit

Subject	Time	Procedure	Blood citrate
			mg. per cent
Rabbit D	2.40 p.m.	1.0 gm. $NaHCO_3$ per kilo	
	4.15 "	Amytal anesthesia	
	5.50–6.20 p.m.	Blood taken from	
		Portal vein	15.0
		Hepatic "	15.0
		Renal "	12.0
		Hepatic "	15.0
		Heart	15.0
Rabbit E	1.12 p.m.	1.0 gm. citric acid per kilo	
	1.30 "	Amytal anesthesia	
	2.37–2.49 p.m.	Blood taken from	
		Heart	21.0
		Lower vena cava	22.0
		Portal vein	25.0
		Hepatic "	25.0
		Heart	23.0

small in comparison with the large decrease caused by fasting. This is in agreement with the observations on human urine showing that alimentary processes as well as acid-base relationships are concerned in citric acid production.

In an attempt to localize citrate production and metabolism in particular organs of the body, a number of experiments were performed in which the citrate content of blood taken from various veins was compared with that taken from the heart. Rabbit D (Table III) was given 1.0 gm. of sodium bicarbonate per kilo by stomach tube and anesthetized with sodium amytal. Blood

samples taken from the portal and hepatic veins and from the heart all contained 15 mg. per 100 cc.; no detectable changes in citrate content occurred in passing through the liver or the intestinal circulation. Blood taken from the renal vein contained only 12 mg., indicating that the kidney was removing citrate from the general circulation. After a similar experiment in which blood leaving the kidney was found to contain more citrate than that taken from the heart, Östberg (15) suggested that citrate is made in the kidney. His analyses may have been unreliable because his data show that inorganic salts interfered with his citrate determinations. In another experiment (Rabbit E) samples of blood

TABLE IV

Influence of Exercise and Food on Citrate Content of Human Blood

Time	Procedure	Blood citrate
		mg. per cent
12.10 p.m.	Mixed lunch	
3.10 "	Blood taken from arm	2.8
3.16 "	2 minutes strenuous exercise	
3.25 "	Blood taken from arm	2.8
5.00 "	" " " "	2.4
9.48 a.m.	5 gm. $NaHCO_3$ (fasting)	
11.40 "	Blood taken from right arm after vigorous working of fist	2.0
11.50 "	Blood taken from resting left arm	2.0

taken after the administration of citric acid showed slightly larger amounts of citrate present in the portal and hepatic veins than in the rest of the circulation, probably due to absorption from the intestine, but, as with Rabbit D, the values do not differ sufficiently to relate any organ to citrate metabolism. They rather suggest, as does also the discovery of a citric acid dehydrogenase in muscle (18), that all active tissue may be involved.

Finally, Table IV shows the influence of exercise and of food on the citrate content of human blood. A sample taken from the arm 3 hours after lunch contained 2.8 mg. of citric acid per 100 cc., a second sample taken 10 minutes after a 2 minute period of strenuous exercise which markedly lowered the citrate content of the urine, contained the same amount. The citrate content of a third

sample, taken an hour and a half later, had dropped to 2.4 mg. per 100 cc. The following morning, the subject ate no breakfast but took 5 gm. of sodium bicarbonate. A sample of blood taken 2 hours later from the right arm after the muscles of the forearm had been thoroughly tired out by opening and closing the fist, contained the same amount of citrate as a second control sample taken from the resting left arm, 2 mg. per 100 cc. Apparently, the rapid decrease in urine citrate due to exercise is not a reflection of a marked change in blood citrate. Sensitive as the enzyme method is, at levels as low as 2 mg. per 100 cc., it has too large a limit of error to detect with certainty the decrease in blood citrate necessary to account for the lower rate of excretion by the kidney. The changes in the blood citrate from 2.8 mg. per 100 cc. shortly after lunch to 2.4 mg. just before dinner and 2.0 mg. during a morning's fast suggest that urinary citrate following meals may reflect the concentration of citric acid in the blood, but there is as yet no information as to what the nature of the kidney threshold for citrate may be. Perhaps it is subject to variations similar to those of uric acid (16).

SUMMARY

Alkalosis and acidosis produced in rabbits by sodium bicarbonate and ammonium chloride administration increased and decreased respectively the serum citrate concentration; inanition produced a marked decrease.

The citric acid content of human urine increased slightly after each meal irrespective of the presence of citric acid in the food. Alkalosis produced by hyperpnea increased citrate excretion; vigorous exercise and the ingestion of ammonium chloride decreased it. Evidence is presented to show that the variations following the intake of food cannot be explained solely on the basis of acid-base relationships.

Citric acid is rapidly but not completely oxidized by the human organism; when given in doses of 2 to 20 gm., 1.5 to 2.5 per cent escaped oxidation and was excreted in the urine.

Analyses of rabbit blood drawn from various parts of the body failed to demonstrate a relation between any particular tissue and the oxidation of citric acid. Blood from the renal vein contained less citric acid than did blood from the renal artery.

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